

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application:

LISTING OF CLAIMS:

1.-155. (canceled).

156. (new) A method of making a live attenuated bacterial vector vaccine, comprising transforming a bacterial strain with an expression vector, wherein said expression vector comprising a nucleotide sequence encoding:

a restricted-copy-number origin of replication cassette comprising (i) a nucleotide sequence encoding an origin of replication that limits the expression vector to an average plasmid copy number of about 2 to 75 copies per cell, (ii) a first unique restriction enzyme cleavage site located 5' of the nucleotide sequence encoding the origin of replication, and (iii) a second unique restriction enzyme cleavage site located 3' of the nucleotide sequence encoding the origin of replication;

at least one post-segregational killing cassette comprising (i) a nucleotide sequence encoding at least one post-segregational killing locus, (ii) a first unique restriction enzyme cleavage site located 5' of the nucleotide sequence encoding the at least one post-segregational killing locus, and (iii) a second unique restriction enzyme cleavage site located 3' of the nucleotide sequence encoding the at least one post-segregational killing locus; and

at least one partitioning cassette comprising (i) a nucleotide sequence encoding at least one partitioning function, (ii) a first unique restriction enzyme cleavage site 5' of the nucleotide

sequence encoding the at least one partitioning function, and (iii) a second unique restriction enzyme cleavage site located 3' of the nucleotide sequence encoding the at least one partitioning function.

157. (new) The method of claim 156, wherein the restricted-copy-number origin of replication is selected from the group consisting of: *oriEl* (nucleotides 1250 to 1936 of SEQ ID NO: 1), *ori101* (nucleotides 50 to 2004 of SEQ ID NO: 3), and *ori15A* (nucleotides 50 to 684 of SEQ ID NO: 2).

158. (new) The method of claim 156, wherein the average plasmid copy-number falls within the range of about 5 to about 60 copies per cell.

159. (new) The method of claim 156, wherein the nucleotide sequence encoding the at least one post-segregational killing locus is selected from the group consisting of *asd*, *ssb*, *phd-doc*, *kis-kid*, and *hok-sok*.

160. (new) The method of claim 156, wherein the partitioning function is an active partitioning function.

161. (new) The method of claim 156, wherein the nucleotide sequence encoding the at least one partitioning function comprises *parA*.

162. (new) The method of claim 156, wherein the partitioning function is a passive partitioning function.

163. (new) The method of claim 156, wherein the nucleotide sequence encoding the at least one partitioning function is the *par* locus of pSC101.

164. (new) The method of claim 156, further comprising an expression cassette comprising (i) a nucleotide sequence encoding a promoter, (ii) a first unique restriction enzyme cleavage site located 5' of the nucleotide sequence encoding the promoter, and (iii) a second unique restriction enzyme cleavage site located 3' of the nucleotide sequence encoding the promoter.

165. (new) The method of claim 164, wherein the promoter is an inducible promoter.

166. (new) The method of claim 165, wherein the promoter is an *ompC* promoter.

167. (new) The method of claim 166, wherein the *ompC* promoter is a polynucleotide fragment from *E. coli* spanning nucleotides +70 through -389, relative to the transcriptional start site +1, of *ompC*.

168. (new) The method of claim 166, wherein the *ompC* promoter comprises the following sequence: AGATCX¹X²TAAAX³CATCCACAGGAGGATATCTGATG (SEQ ID NO:36), wherein X¹ is selected from the group consisting of G, C and A; X² is an insert having from 1 to 5 nucleotides; and X³ is selected from the group consisting of A, T, G and C.

169. (new) The method of claim 168, wherein X¹ is G.

170. (new) The method of claim 168, wherein X² has from 1 to 4 nucleotides.

171. (new) The method of claim 168, wherein X² has 4 nucleotides.

172. (new) The method of claim 168, wherein X² has 4 nucleotides, independently selected from the group consisting of A, T and C.

173. (new) The method of claim 168, wherein X² comprises a nucleotide or nucleotide sequence selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

174. (new) The method of claim 168, wherein X² is selected from the group consisting of ATCT; ATC; AT; TCT; CT; TC; A; T; C; and T.

175. (new) The method of claim 168, wherein X² is ATCT.

176. (new) The method of claim 168, wherein X³ is A.

177. (new) The method of claim 164, wherein the expression cassette further comprises a nucleotide sequence encoding an antigen of interest located at the 3' end of nucleotide sequence encoding the promoter.

178. (new) The method of claim 177, wherein the antigen of interest is selected from the group consisting of a viral antigen, a bacterial antigen, a cancer antigen, and an auto-immune antigen.

179. (new) The method of claim 177, wherein the antigen of interest comprises a detoxified Shiga toxin.

180. (new) The method of claim 179, wherein the antigen of interest comprises a detoxified Shiga toxin 2 antigen selected from the group consisting of a Shiga toxin 2 B subunit pentamer and a genetically detoxified Shiga toxin 2.

181. (new) The method of claim 180, wherein the gene encoding the detoxified Shiga toxin 2 has modified segments selected from the group consisting of:

(797) - ACA GCA GAC GCG TTA - (811) (SEQ ID NO: 37);

(902) - CTG AAC CTA GGG CGA (916) (SEQ ID NO: 38);

(1345) - GAA TTC GCG ACC AGT - (1359) (SEQ ID NO: 39) and

(1435) - GAA TCA GAT TCT GGA - (1449) (SEQ ID NO: 40).

182. (new) The method of claim 156, further comprising a selection cassette comprising (i) a nucleotide sequence encoding at least one selectable marker, (ii) a first unique restriction enzyme cleavage site located 5' of the nucleotide sequence encoding the at least one selectable marker, and (iii) a second unique restriction enzyme cleavage site located 3' of the nucleotide sequence encoding the at least one selectable marker.

183. (new) The method of claim 182, wherein the selectable marker is a protein which provides resistance to an antibiotic selected from the group consisting of aminoglycosides, ansamycins, antimycotics, penicillins, cephalosporins, chloramphenicols, linosamides, macrolides, peptolides, and tetracyclines.

184. (new) The method of claim 182, wherein the nucleotide sequence encoding the selectable marker is selected from the group consisting of *tetA*, *bla*, *aphA-2*, and *kan*.

185. (new) The method of claim 156, wherein the bacterial strain is a prokaryotic cell.

186. (new) The method of claim 185, wherein the prokaryotic cell is *Salmonella typhi*.

187. (new) The method of claim 185, wherein the prokaryotic cell is a *Salmonella typhi* strain.